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(54) Title: METHODS OF USING CHEMICAL LIBRARIES TO SEARCH FOR NEW KINASE INHIBITORS

(57) Abstract

The generation of selective inhibitors for specific protein kinases would provide new tools for analyzing signal transduction pathways and possibly new therapeutic agents. We have invented an approach to the development of selective protein kinase inhibitors based on the unexpected binding mode of 2,6,9–trisubstituted purines to the ATP binding site of human CDK2. The most potent inhibitor, purvalanol B (IC_{50} = 6 nM), binds with a 30–fold greater affinity than the known CDK2 inhibitor, flavopiridol. The cellular effects of this class of compounds were examined and compared to those of flavopiridol by monitoring changes in mRNA expression levels for all genes in treated cells of *Saccharomyces cerevisiae* using high–density oligonucleotide probe arrays.

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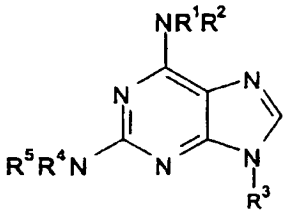
WHAT IS CLAIMED IS:

1. A method of identifying compounds which modulate cell proliferation, said method comprising treating at least one cell with at least one compound, isolating a plurality of mRNA transcripts from the cell by hybridizing under stringent conditions to at least one oligonucleotide complementary to a nucleic acid sequence which encodes a protein associated with cell proliferation, and comparing the plurality of mRNA transcripts from said cell to a plurality of mRNA transcripts from a genetically identical cell not treated with the compound and isolated by hybridization under stringent conditions to the oligonucleotide, whereby a difference in the number of mRNA transcripts from the treated and untreated cells hybridized to the oligonucleotides indicates a modulation of cell proliferation.

2. The method of claim 1, wherein the compounds are inhibitors of cyclin-dependent kinases.

3. The method of claim 2, wherein the inhibitors have the following structure:



R^1, R^2, R^3, R^4 and R^5 are independently members selected from the group consisting of H, C_1-C_8 straight-chain, branched-chain, saturated and unsaturated alkyl, C_1-C_8 straight-chain, branched-chain, saturated and unsaturated substituted alkyl, aryl and substituted aryl.

4. The method of claim 1, wherein the mRNA transcripts are cRNA.

5. The method of claim 1, further comprising isolating mRNA transcripts of treated and untreated cells which encode proteins associated with cell proliferation.

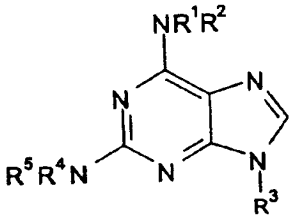
6. The method of claim 1, wherein the oligonucleotides are from 15 to about 50 nucleotides in length.

7. The method of claim 1, wherein the oligonucleotides are linked to a solid support in a high density array.

8. A method of determining the identity of proteins that modulate cell proliferation during or after exposure to chemical or genetic challenges, said method comprising isolating mRNA transcripts generated from cells after exposure to compounds known to modulate cellular proliferation by hybridizing under stringent conditions to at least one oligonucleotide complementary to a nucleic acid sequence which encodes a protein associated with cell proliferation, isolating mRNA transcripts generated from cells not exposed to said compounds by hybridizing to the oligonucleotides, comparing the total number of mRNA transcripts from both treated and untreated cells, and determining which proteins are encoded by mRNA transcripts present in differing amounts in treated or untreated cells.

9. The method of claim 8, wherein the compounds are cyclin-dependent kinase inhibitors.

10. The method of claim 9, wherein the inhibitors have the following structure:



R^1, R^2, R^3, R^4 and R^5 are independently members selected from the group consisting of H, C_1-C_8 straight-chain, branched-chain, saturated and unsaturated alkyl, C_1-C_8 straight-chain, branched-chain, saturated and unsaturated substituted alkyl, aryl and substituted aryl.

11. The method of claim 8, wherein the mRNA transcripts are cRNA.

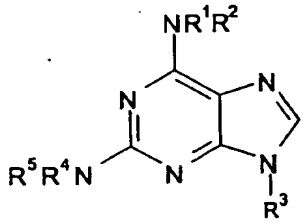
12. The method of claim 8, wherein oligonucleotides are about 15 to about 50 nucleotides in length.

13. The method of claim 12, wherein the oligonucleotides are linked to a solid support in a high density array.

14. A method of determining proteins associated with increased drug resistance, said method comprising isolating mRNA transcripts generated from drug-resistant cells after exposure to drugs known to inhibit cellular proliferation by hybridizing under stringent conditions to at least one oligonucleotide complementary to a nucleic acid sequence which encodes a protein associated with cell proliferation, isolating mRNA transcripts generated from non-drug resistant cells exposed to said drugs by hybridizing under stringent conditions to the oligonucleotides, comparing the total number of mRNA transcripts from both drug-resistant and drug non-resistant cells, and determining which proteins are encoded by mRNA transcripts present in increased amounts in the drug-resistant cells.

15. The method of claim 14, wherein the compounds are cyclin-dependent kinase inhibitors.

16. The method of claim 15, wherein the inhibitors have the following structure:



R^1, R^2, R^3, R^4 and R^5 are independently members selected from the group consisting of H, C_1-C_8 straight-chain, branched-chain, saturated and unsaturated alkyl, C_1-C_8 straight-chain, branched-chain, saturated and unsaturated substituted alkyl, aryl and substituted aryl.

17. The method of claim 14, wherein the mRNA transcripts are cRNA.

18. The method of claim 14, wherein the oligonucleotides are about 15 to about 50 nucleotides in length.

19. The method of claim 18, wherein the oligonucleotides are linked to a solid support in a high density array.

INTERNATIONAL SEARCH REPORT

Intern. ...al Application No
PCT/US 98/27405

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12Q1/68 A61K31/52 C12Q1/48 //C07D473/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12Q A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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Y	WO 95 28169 A (UNIV CALIFORNIA) 26 October 1995 see page 1 - page 3 ---	1-19

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[X] Further documents are listed in the continuation of box C.

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